Self-aggregation of Synthetic Zinc 3-Hydroxymethyl-purpurin-18 and *N*-Hexylimide Methyl Esters in an Aqueous Solution as Models of Green Photosynthetic Bacterial Chlorosomes

Hitoshi Tamiaki,* Yasuhide Shimamura, Hideaki Yoshimura, Suresh K. Pandey,† and Ravindra K. Pandey†
Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525-8577
†*PTD Center, Roswell Park Cancer Institute, Buffalo, New York 14263, U. S. A.*

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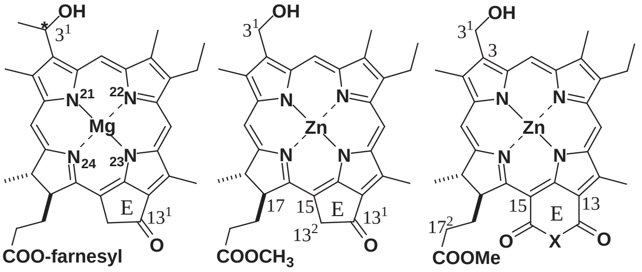
Zinc 3^1-hydroxy-13^1-oxo-chlorins possessing a six-membered exo-ring attached at the 13- and 15-positions were prepared by modifying purpurin-18. These synthetic anhydride and imide self-aggregated in an aqueous THF solution to give large oligomers (J-aggregates) similarly with the corresponding natural and artificial chlorophylls possessing a five-membered E-ring.

Self-assemblies of π-conjugated moieties attract much attention from the viewpoints of chemistry, biology, and material science.[1] Such assemblies are observed in various natural systems, especially (bacterio)chlorophylls [= (B)Chls] stacked in photosynthetic organisms. X-ray crystallographic structure analyses of some (B)Chl-containing proteins have clearly shown π–π stacking of (B)Chls.[2] On the other hand, self-aggregates of chlorophyllous pigments are found in main light-harvesting antennas of green photosynthetic bacteria (= chlorosomes).[3] The composite Chls are characterized by 3^1-hydroxy and 13^1-oxo groups as shown in the left drawing of Figure 1. As one of the model pigments, zinc 3^1-hydroxy-13^1-oxo-chlorin **3** (middle drawing of Figure 1) was reported and self-aggregated similarly under various conditions.[4] All natural and artificial chlorosomal Chls had a five-membered exo-ring (E-ring) and a keto (or formyl) carbonyl group on the Q_y axis (N21–N23) has been requisite for chlorosomal self-aggregation so far.[5] Here, we report the synthesis of zinc 3^1-hydroxy-13^1-oxo-chlorins **1** and **2** (right drawing of Figure 1) where the 15-methylene group of **3** was formally substituted with –COO– and –CON(C_6H_{13})–, respectively, and chlorosome-like self-aggregation of synthetic anhydride **1** and imide **2** possessing a six-membered exo-ring in an aqueous solution. An aqueous solution containing 6% (v/v) THF was reported to be one of the effective environments for self-aggregation of chlorosomal Chls[6–8] and was used in this study.



BChl-*d* **3** **1** (**X =O**) **2** (**X = NC_6H_{13}**)

Figure 1. Molecular structures of a naturally occurring chlorosomal Chl, one of BChl-*d* homologs (left), its synthetic model **3** (middle), and **1**/**2** (right).

Purpurin-18 was afforded by air-oxidation[9] of pheophorbide-*a* prepared from Chl-*a* (see Supporting Information for the present synthetic route).[10] After methyl esterification of the 17^2-carboxy group,[11] the 3-vinyl group was oxidatively cleaved,[12] the resulting 3-formyl group was selectively reduced,[12] and then zinc-metallation[12] gave desired zinc 3-hydroxymethyl-purpurin-18 methyl ester **1**.[13] The anhydride moiety of purpurin-18 methyl ester was transformed to the corresponding *N*-hexylimide[11] and the imide was modified similarly as the above synthesis of **1** to yield zinc 3-hydroxymethyl-purpurin-*N*-hexylimide methyl ester **2**.[13] Reference compound **3**, zinc 3-hydroxymethyl-pyropheophorbide-*a* methyl ester was prepared according to reported procedures.[4]

In THF, anhydride **1** formed monomeric 5-coordinated zinc species with a THF molecule as an axial ligand to give sharp and intense Q_y and Soret bands at 665 and 421 nm, respectively (broken line of Figure 2A upper). The Q_y peak was shifted to a 9-nm longer wavelength than that of keto-type **3** (646 nm, see Table 1), which was due to the substituent effect of the E-ring on the Q_y axis. Such a THF solution containing monomeric **1** was diluted with water and broad absorption bands appeared in the red-shifted regions. In 6% (v/v) THF–water, a Q_y maximum was observed at 753 nm (solid line of Figure 2A upper),[14] which was a 1760 cm^{-1} bathochromic shift compared with the monomeric (see Table 1). In the red-shifted regions, intense CD bands were recorded, while the monomeric **1** gave smaller CD peaks (Figure 2A lower). These changes (broadening and red-shift in electronic absorption bands and enlargement in CD bands) were observed in natural chlorosomes and their artificial models,[3,4] indicating that **1** self-aggregated in the aqueous solution to form similar large oligomers (J-aggregates). Moreover, the shape of broadened and red-shifted Q_y band in self-aggregates of **1** and the reverse S-shape of the pronounced CD band at the red-shifted Q_y region are almost the same as those in **3** (see Supporting Information), and the red-shift value in **1** is comparable to that of **3** (1780 cm^{-1}). In the self-aggregation, the anhydride moiety in the E-ring of **1** functioned as the keto-carbonyl moiety in the E-ring of chlorosomal Chls and their model pigments including BChl-*d* and **3**. The 13-carbonyl group in the anhydride moiety on the Q_y

Table 1. Q_y maxima λ_{max} (nm) and the red-shifts Δ (cm^{-1}) by self-aggregation

	λ_{max}[a]		
	THF	6%(v/v)THF–water	Δ[b]
1 (anhydride)	665	753	1760
2 (imide)	671	729	1190
3 (ketone)	646	730	1780

[a] In ca. 5 μM. [b] $\Delta = [1/\lambda_{max}(\text{THF}) - 1/\lambda_{max}(6\%\text{THF–water})] \times 10^7$.

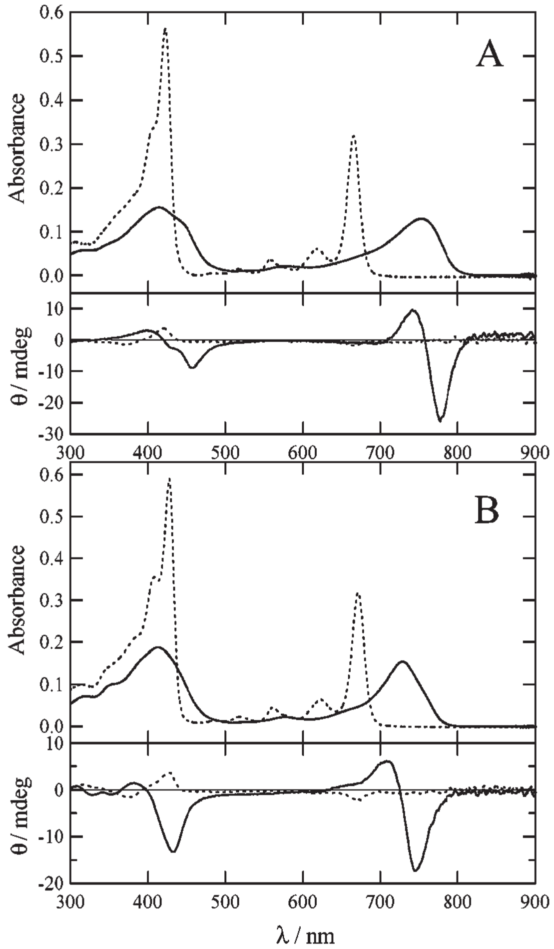


Figure 2. Electronic (upper) and CD absorption spectra (lower) of ca. 5 μM solutions of **1** (A) and **2** (B) in THF (broken) and 6% (v/v) THF–water (solid).

axis would hydrogen-bond intramolecularly with zinc-coordinated 3^1-hydroxy group to form highly ordered supramolecules as well as the keto-carbonyl groups did.[3,4] The 15-carbonyl group of **1** parallel to the Q_x axis (N22–N24) would not disturb the self-aggregation, which is consistent with the fact that no 7-formyl group could perturb self-aggregation in natural and artificial systems.[7,15]

Imide **2** was monomeric in THF and gave a sharp 671-nm Q_y maximum (broken line of Figure 2B upper), which was shifted to a longer wavelength than those of **1** and **3**. In 6% (v/v) THF–water, **2** also self-aggregated to afford a broadened and red-shifted Q_y band at 729 nm[14] and a reverse S-shaped CD couplet (solid lines of Figure 2B). The Q_y peak position in self-aggregates of **2** was comparable to that of **3** and shifted to a 24-nm shorter wavelength than that of **1**. The value in the red-shift by self-aggregation of **2** was 1190 cm^{-1} and smaller than those of **1** and **3**. Although supramolecule-forming motifs (13-C=O···H–O···Zn and π–π interaction)[3,4] would be the same among self-aggregates of **1**–**3**, imide **2** slightly disturbed the intermolecular interaction to give a less red-shifted Q_y band in self-aggregates than anhydride **1** and keto **3**. The slight suppression in **2** was ascribed to the steric interference of the hexyl group on the imide nitrogen neighbor to the interactive 13-carbonyl group. This interpretation was consistent with the fact that self-aggregates of 13^2-methoxycarbonylated **3** (zinc 3-hydroxymethyl-pheophorbide-*a* methyl ester) gave a less red-shifted Q_y band at 697 nm ($\Delta = 1230\,\mathrm{cm}^{-1}$).[6] It is noteworthy that 3^1-methylated **3** (zinc bacteriopheophorbide-*d* methyl ester) gave a similar red-shift value (1200 cm^{-1}) by its self-aggregation.[8] In self-aggregative pigments, therefore, six-membered anhydride and imide rings fused at 13- and 15-positions could be alternated for the five-membered E-ring possessing a keto-carbonyl group as seen in all natural chlorosomal Chls and their model pigments; anhydride and imide carbonyl groups as well as keto-C=O are effective for chlorosomal self-aggregation.

As reported earlier,[11] anhydride- and imide-type Chls as in **1** and **2** were more stable in their monomeric states than the corresponding keto-type as in **3**, while all the self-aggregates of **1**–**3** were fairly stable even in an aerated solution under irradiation with visible light. Such chemical stabilities of anhydrides and imides are advantageous for preparation of self-aggregative pigments and their further modification. The hexyl group on the imide nitrogen of **2** slightly suppressed the self-aggregation but could still construct a similar large oligomer. Any functional groups can be easily substituted on the nitrogen as in **2**[11,16] and various functions including energy-donating and accepting groups would be introduced to self-aggregates of imides. Preparation of such functional supramolecules is now in progress.

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References and Notes

1 F. J. M. Hoeben, P. Johkheijm, E. W. Meijer, and A. P. H. J. Schenning, *Chem. Rev.*, **105**, 1869 (2005).
2 T. Oba and H. Tamiaki, *Bioorg. Med. Chem.*, **13**, (2005), in press.
3 H. Tamiaki, *Coord. Chem. Rev.*, **148**, 183 (1996); J. M. Olson, *Photochem. Photobiol.*, **67**, 61 (1998).
4 H. Tamiaki, M. Amakawa, Y. Shimono, R. Tanikaga, A. R. Holzwarth, and K. Schaffner, *Photochem. Photobiol.*, **63**, 92 (1996).
5 S. Yagai, T. Miyatake, and H. Tamiaki, *J. Org. Chem.*, **67**, 49 (2002).
6 T. Oba and H. Tamiaki, *Photosynth. Res.*, **61**, 23 (1999).
7 H. Tamiaki, M. Kubo, and T. Oba, *Tetrahedron*, **56**, 6245 (2000).
8 S. Sasaki and H. Tamiaki, *Bull. Chem. Soc. Jpn.*, **77**, 797 (2004).
9 A. S. Brandis, A. N. Kozyrev, and A. F. Mironov, *Tetrahedron*, **48**, 6485 (1992).
10 N. Kosaka and H. Tamiaki, *Eur. J. Org. Chem.*, **2004**, 2325.
11 G. Zfeng, W. R. Potter, S. H. Camacho, J. R. Missert, G. Wang, D. A. Bellnier, B. W. Henderson, M. A. J. Rodgers, T. J. Dougherty, and R. K. Pandey, *J. Med. Chem.*, **44**, 1540 (2001).
12 H. Tamiaki, S. Miyata, Y. Kureishi, and R. Tanikaga, *Tetrahedron*, **52**, 12421 (1996).
13 All zinc complexes **1**–**3** were purified with HPLC and identified from visible absorption, ^{1}H NMR and MS spectra. See Supporting Information for spectral data of **1** and **2**.
14 In 6%(v/v)THF–water, ca. 1 μM solutions of **1** and **2** gave the same shapes of electronic absorption bands as in the solid lines of Figures 2A and 2B upper (ca. 5 μM), respectively.
15 H. Tamiaki, *Photochem. Photobiol. Sci.*, **4**, (2005), in press.
16 Substitution at the 7- and 8-positions and the 17-propionate on the Q_x axis of a (bacterio)chlorin moiety were also useful for such modification; see Refs. 15 and 17.
17 T. Miyatake, H. Tamiaki, A. R. Holzwarth, and K. Schaffner, *Photochem. Photobiol.*, **69**, 448 (1999); T. Miyatake, H. Tamiaki, M. Fujiwara, and T. Matsushita, *Bioorg. Med. Chem.*, **12**, 2173 (2004).

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